The Quantum Yield of Photosynthesis in *Porphyridium cruentum*, and the Role of Chlorophyll *a* in the Photosynthesis of Red Algae

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ABSTRACT Quantum yield measurements were made with the red alga *Porphyridium cruentum*, cultured so as to give different proportions of chlorophyll and phycobilins. Totally absorbing suspensions were used so that there was no uncertainty in the amount of energy absorbed. These measurements have shown that chlorophyll, in this alga, has a photosynthetic efficiency as high as in other algal groups, and higher than the phycobilins—at least at wave lengths shorter than about 650 mμ. Wave lengths longer than this are beyond the range of maximum efficiency of chlorophyll. Under specified conditions of temperature and supplementary light full efficiency may be extended to longer wave lengths. The results of these measurements have made it unnecessary to suppose that in red algae chlorophyll plays a minor role while the phycobilins are the photosynthetic sensitizers of primary importance.

Comparison of the responses of different classes of algae to light of different wave lengths has led to speculation that in red algae the chlorophyll *a* might play a relatively minor part in photosynthesis, or that some of the chlorophyll might be inactive, and that the light absorbed by the inactive fraction would be ineffective in photosynthesis. Haxo and Blinks (1950) were the first to notice differences between the red algae and algae with other combinations of pigments. They measured action spectra for photosynthesis, with green, brown, and red algae. Green and brown algae showed a maximum in photosynthetic activity corresponding with the red absorption maximum of chlorophyll, while red algae showed relatively low activity in wave lengths strongly absorbed by chlorophyll, and a maximum in photosynthetic activity corresponding with the absorption maximum of phycoerythrin in the green.

Yocum and Blinks (1954) made direct measurements of the quantum yield of photosynthesis as a function of wave length for various red algae, and found low yields near the absorption maximum of chlorophyll, and higher yields

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where most of the light was absorbed by phycoerythrin, in confirmation of the conclusions drawn by Haxo and Blinks from action spectra. Duysens (1952) measured yield of photosynthesis with the red alga *Porphyridium cruentum*, and obtained results similar to those reported by Yocum and Blinks.

Blinks and coworkers sought to interpret their results by suggesting that in red algae the chlorophyll *a* might play a less direct part in the sensitization of photosynthesis than the primary role assigned to it in the case of other photosynthetic algae and higher plants, and that in red algae phycoerythrin might be the sensitizer of primary importance. Duysens, on the other hand, having observed that chlorophyll fluorescence could be excited through absorption of light by phycoerythrin, and having found evidence that transfer of excitation energy from phycoerythrin to chlorophyll could take place with very small losses, thought it likely that in red algae, chlorophyll *a* remains the direct sensitizer of photosynthesis, just as it is in other algae and green plants. He introduced certain *ad hoc* assumptions to account for the relatively low yield of photosynthesis for light absorbed by the chlorophyll of red algae. He suggested that in red algae the chlorophyll might be present in two forms, one of which was prone to lose its excitation energy easily by resonance transfer to another pigment (presumably chlorophyll *d*). The other form he supposed to be protected in some way (possibly because of its spatial arrangement) against loss of excitation energy through this channel. He suggested that the excitation energy passing to chlorophyll from phycoerythrin might go preferentially to those chlorophyll molecules protected against transfer to chlorophyll *d*, and hence might be used efficiently for photosynthesis, while light directly absorbed by chlorophyll would be lost for photosynthesis to the extent that it was absorbed by molecules prone to transfer their excitation energy to chlorophyll *d*.

Although there is room for doubt about the precision of measurements of the scattered light transmitted by pieces of algal thallus or suspensions of *Porphyridium* cells, (and such measurements enter into the estimation of yields of photosynthesis in the experiments of Duysens, as well as those of Blinks and coworkers), there can be no question about the relatively low yield for light absorbed by chlorophyll near its red absorption maximum compared to the high yield for light absorbed by phycoerythrin near its maximum in the green. Chlorophyll accounts for practically all the absorption of the longer wave lengths of red, while in the green the phycoerythrin may account for as much as 95 per cent of the absorption. With such extreme differences in distribution of absorption between pigments, possible errors in the estimation of absorbed energy could hardly modify the general conclusion about the relatively low efficiency of the light absorbed by the chlorophyll (at least at the longer wave lengths where all absorption is by chlorophyll). On the other hand, between the absorption maxima of chlorophyll and phycoerythrin is a range of wave

lengths where absorption is divided between chlorophyll and phycobilins in varying proportions. In this region, the conclusions to be drawn from the measurements of Duysens, and of Blinks and coworkers, are less certain, because the fraction of the incident light absorbed by the cells is smaller, and is therefore determined with less precision. Yocum (1951) drew attention to the greater uncertainty of the quantum yield in regions in which absorption was small, and Duysens (1952, p. 67) mentions especially the uncertainty about the yield at 630 mμ.

The measurements of Duysens, and of Blinks and coworkers, therefore, provide a somewhat uncertain basis for estimating the yield of photosynthesis between absorption maxima, and it is impossible to decide from their measurements whether the yield for the light absorbed by chlorophyll is as low in these intermediate wave lengths as it is near the red absorption maximum of chlorophyll.

Because of the ease with which the cadmium line at 644 mμ can be isolated with reasonable intensity and spectral purity, we used that wave length for many measurements of the quantum yield of *Porphyridium*. At 644 mμ absorption is divided more or less evenly between chlorophyll and phycocyanin (see below).

For comparison, we also made measurements with the green and blue mercury lines (546 and 436 mμ respectively) which were easily isolated with satisfactory intensity by means of filters. At 546 mμ about 95 per cent of the absorption is attributable to β-phycoerythrin. At 436 mμ the absorption is divided between chlorophyll, carotenoids, and phycoerythrin in uncertain proportions.

We found that in general *Porphyridium* cells showed considerably higher yields of photosynthesis at 644 mμ than at 546 mμ. Cells grown in green light (Brody and Emerson, 1959) were an exception, and showed yields nearly as high at 546 as at 644 mμ. The quantum yield at 644 mμ was relatively constant for cells grown under all conditions of illumination, close to 0.09. This is in agreement with yields reported by various authors for light absorbed by chlorophyll in many types of algae. It does not suggest that the chlorophyll of *Porphyridium* is less active than it is in other algae, but rather that in *Porphyridium* (and perhaps in red algae generally) there must be a substantial drop in the quantum yield of photosynthesis between 644 mμ and the region 675 to 685 mμ, in which Blinks and coworkers, and also Duysens, found evidence of exceptionally low yields. We thought it necessary to measure quantum yields between 644 and 685 mμ, under conditions of total absorption of the incident light, so that uncertainty about the fraction of light absorbed by the cells could be excluded as a source of uncertainty in the quantum yields. We made these comparisons with *Porphyridium cells* cultured so as to give different proportions of chlorophyll and phycoerythrin

(Brody and Emerson), and we extended the quantum yield measurements to the violet end of the visible spectrum, though the yields in blue and violet were of low precision because of the small light output of the lamp and the consequent use of wide band widths.

The results of these measurements are reported here, together with our conclusions concerning the contribution of chlorophyll and phycoerythrin to the photosynthesis of *Porphyridium*.

Materials and Methods

Of the red algae, the unicellular *Porphyridium cruentum* is the species best suited for studying photosynthesis by the manometric methods developed for the green alga *Chlorella*. Blinks and coworkers arrived at their conclusions mostly from experiments with multicellular red algae, but since Duysens obtained essentially the same or very similar results with *Porphyridium*, we believe that comparisons between our results and those of Blinks and coworkers are significant for purposes of estimating the contributions of chlorophyll and phycoerythrin to photosynthesis, in spite of the different species used.

The use of unicellular algae permits control of light absorption by adjustment of suspension density. The chief source of uncertainty in the experiments of Blinks and coworkers, and also in those of Duysens, is in the estimation of light absorption. Their experiments were all made with algal material which transmitted appreciable fractions of the incident light. The absorbed energy, whether in relative or absolute units, was derived by subtracting the transmitted from the incident energy. Methods are available for measurement of incident light with adequate precision, but the measurement of diffusely transmitted light requires the use of integrating devices which involve various approximations, and their precision is not easily estimated. As we mentioned in the introduction, errors may not be important if the fraction of light absorbed is large, but if the absorbed fraction is small, then small errors in the estimation of the transmitted light lead to much larger errors in the estimated absorption. Thus the uncertainty in quantum yields is greater, the smaller the fraction of incident light absorbed. This source of uncertainty can be avoided, when working with suspensions of algal cells, by making the suspension density so great that at all wave lengths the transmitted fraction is close to zero. Then for practical purposes, I_0 (incident) becomes equal to I (absorbed). Our measurements were made with cell suspensions sufficiently dense to fulfill this approximation, thus avoiding the problem of integrating scattered light.

For our earlier experiments the incident beam was from a 400 watt mercury-cadmium lamp (North-American Philips Co.), from which we isolated the red cadmium line of wave length 644 mμ, the green mercury line at 546 mμ, or the blue mercury line at 436 mμ, by means of filters. The combination of a heat-absorbing glass (American Optical Company) and a sharp cut-off red glass (Corning Glass Company) gave a beam of red light which contained no wave lengths shorter than 644 mμ. Eighteen per cent of the energy was in wave lengths longer than 644 mμ.

These longer wave lengths were for the most part longer than 700 mμ, and contributed nothing to photosynthetic activity. For calculation of quantum yields, we included only the energy attributable to the 644 mμ line (about 1.5 micro-einsteins per minute). The combination of filters used to isolate the green and blue lines transmitted no infrared.

Later we made measurements with bands of wave lengths from a grating monochromator (the same instrument that had been built by Emerson and Lewis (1943), aperture f 1.5). The light source was a 30 ampere tungsten ribbon filament lamp operated at 7.5 volts. This arrangement provided about 1 micro-einstein per minute with band widths ranging from about 16 mμ in the red to about 50 mμ in the blue and violet.

Details concerning the morphology of the cells (Brody and Vatter, 1959), their cultivation, and proportions of pigments (Brody and Emerson, 1959) have already been published.

For measurements of photosynthesis the cells were centrifuged out of their culture medium and washed three times in carbonate-bicarbonate buffer containing 15.2 gm. sodium chloride per liter. The sodium chloride was added to maintain an osmotic pressure approximating that of the culture medium. Comparison of different carbonate-bicarbonate buffers showed that in saturating white light the highest rates of photosynthesis were attained in mixtures containing higher proportions of bicarbonate than commonly used for experiments with *Chlorella*. For our experiments on the quantum yields of *Porphyridium* we used a buffer containing 5 parts M/10 potassium carbonate, and 95 parts M/10 sodium bicarbonate. This is in equilibrium with approximately 2 per cent carbon dioxide in air. To make up for losses of carbon dioxide resulting from centrifuging, pipetting, etc., a mixture of 2 per cent carbon dioxide in air was flushed through the gas spaces of manometer vessels after they were filled with cell suspension and connected with their manometers.

We used a differential manometer like the one illustrated by Emerson and Chalmers (1955). The manometer vessels were rectangular, and were filled with 7 ml. of cell suspension, leaving a gas space of about 6 ml. We used Brodie solution for manometer fluid (10,000 mm. Brodie = 760 mm. Hg).

Pressure changes were read at intervals of 1 minute, to the nearest hundredth of a millimeter, with horizontal microtelescopes. The pressure changes were read without interruption of shaking. With a shaking frequency of 180 per minute, and an amplitude of 18 mm., 3 minutes sufficed for attainment of rates which were steady within the limit of error of the observations after changes from light to dark or dark to light. Rates of photosynthesis were derived from differences in steady rates in light and darkness, measured over periods of 4 or 5 minutes.

Rates of photosynthesis and respiration were calculated from rates of pressure change on the conventional assumption that the partial pressure of carbon dioxide was maintained constant by the buffer mixture, and that pressure changes were attributable to oxygen exchange alone. We note that Emerson and Chalmers found evidence that in buffer mixture 9 the carbon dioxide pressure changed appreciably, probably between 5 and 10 per cent of the changes in oxygen pressure. In our calculations we made no allowance for this, and our calculated rates of photosynthesis

may be lower than the true rates by as much as 10 per cent, if our buffer mixture permitted exchange of carbon dioxide to the extent estimated by Emerson and Chalmers from their two vessel measurements.

We measured the energy of the incident light beam with a large surface platinum bolometer, which had been calibrated against a radiation standard from the National Bureau of Standards. During calibration, the sensitive surface of the bolometer was protected by a fluorite window, which transmitted 91 per cent of the energy from the radiation standard. For measurement of energy in visible light, a window of crystal quartz was used (transmission for the visible about 91 per cent). Approximate corrections were made for differences in optical path between bolometer surface and cell suspension, on the basis of indices of refraction of the various media.

The shaken manometer vessel intercepted practically the entire area of the beam of incident light at all times, and the density of the cell suspension was adjusted to provide practically total absorption of the incident beam at all wave lengths where yield measurements were made. The quantum yield, Φ, was therefore

$$\Phi = \frac{\text{Oxygen produced by photosynthesis, in micromoles per minute}}{\text{Incident energy, in micro-einsteins per minute}}$$

RESULTS OF EXPERIMENTS

Table I shows the quantum yields of photosynthesis, obtained in preliminary experiments with the red, green, and blue lines isolated from the mercury-cadmium lamp with filters. Data for additional lines are also given, but these are not as significant since the lines were not isolated with great purity. The value for the yield at 578 mμ was obtained by subtracting the effect produced by the 644 mμ line from that produced by both lines.

In general, the yields in green are lower than those in red and increase with increasing wave length—or with increasing fraction of light absorbed by chlorophyll. In the red region the yields approximate a maximum of 0.09, or about 11 quanta of light absorbed per molecule of oxygen produced in photosynthesis. This is close to the maximum yield of photosynthesis reported by various authors for a number of different organisms (Emerson and Lewis, for *Chlorella* (1943), *Chroococcus* (1942), and other algae; Tanada, for the diatom *Navicula minima* (1951); Yuan and coworkers, various algae (1955); Rieke, for *Chlorella* and *Scenedesmus* (1949); Wassink, for various horticultural plants (1946)). Much evidence supports the opinion that this is close to the maximum obtainable, and that reports of higher yields are probably not representative of the chemical change usually understood by the term "photosynthesis" (production of carbohydrates and oxygen from carbon dioxide and water). We may conclude that at 644 mμ the red alga *Porphyridium cruentum* shows a quantum yield of photosynthesis approximating the highest values obtainable with other algae. This implies that at this wave

length, the light absorbed by chlorophyll is probably contributed to photosynthesis at normal efficiency.

Conclusions based on comparisons of yields at 644 mμ with those at 546 are made somewhat less certain than one might desire, by the fact that absorption at 644 is divided not between chlorophyll and phycoerythrin, but mainly between chlorophyll and phycocyanin. If one assumed that the activity of the two phycobilins was the same, a lower efficiency of chlorophyll should begin to manifest itself at about 560 mμ, where the fraction of light absorbed by chlorophyll begins to increase, and the yield at 644 mμ should be *lower* than at both 546 and 625 mμ (the peak of the phycocyanin band).

TABLE I

QUANTUM YIELDS FOR CELLS OF *PORPHYRIDIUM CRUENTUM* GROWN IN DIFFERENT WAVE LENGTHS AND INTENSITIES OF LIGHT

Illumination during last 5 days' growth*	Φ 435 mμ	Φ 460 mμ	Φ 546 mμ	Φ 578 mμ	Φ 578 + 644 mμ	Φ 644 mμ
589 mμ	0.0498	0.0469	0.0775	0.0800	0.0834	0.0876
546 mμ	0.0357	0.0303	0.0870	0.0893	0.0900	0.0910
436 mμ	0.0547	0.0513	0.0555	0.0625	0.0694	0.0870
Low intensity white‡	0.0578	0.0553	0.0645	0.0714	0.0910	0.0926
Medium intensity white‡	0.0505	0.0429	0.0693	0.0704	0.0719	0.0855
High intensity white‡	...	...	0.0771	...	...	0.0848

* See Brody and Emerson for details of culture conditions.

‡ "White" light provided by different numbers of "cool white" fluorescent lamps.

This, as shown by our experiments with the monochromator (to be described later), is definitely not the case. If one should attribute the good yield at 644 mμ entirely to a high efficiency of phycocyanin and assign a low efficiency to chlorophyll, the efficiency of phycocyanin would have to be unreasonably high in relation to that of phycoerythrin (or chlorophyll in other types of algae), since the fractions of light absorbed by chlorophyll and phycocyanin at 644 mμ range from about 70 per cent and 30 per cent respectively in "green light" cells to about 50 per cent and 50 per cent in "blue light" cells (and, of course, the yield at 625 mμ should be higher than at 644 mμ). Therefore, the most probable conclusion from the high yield at 644 mμ is that the light absorbed by chlorophyll is used for photosynthesis at normal efficiency.

Turning again to the yields in green, where some 95 per cent of the light must have been absorbed by phycoerythrin, we see that these are always lower than at 644 mμ, but that for cells grown in green light, the yield in green was generally close to the yield in red. This may be interpreted as evidence that the yield for light absorbed by phycoerythrin may sometimes be nearly as great as the yield for the fraction absorbed by chlorophyll, but that

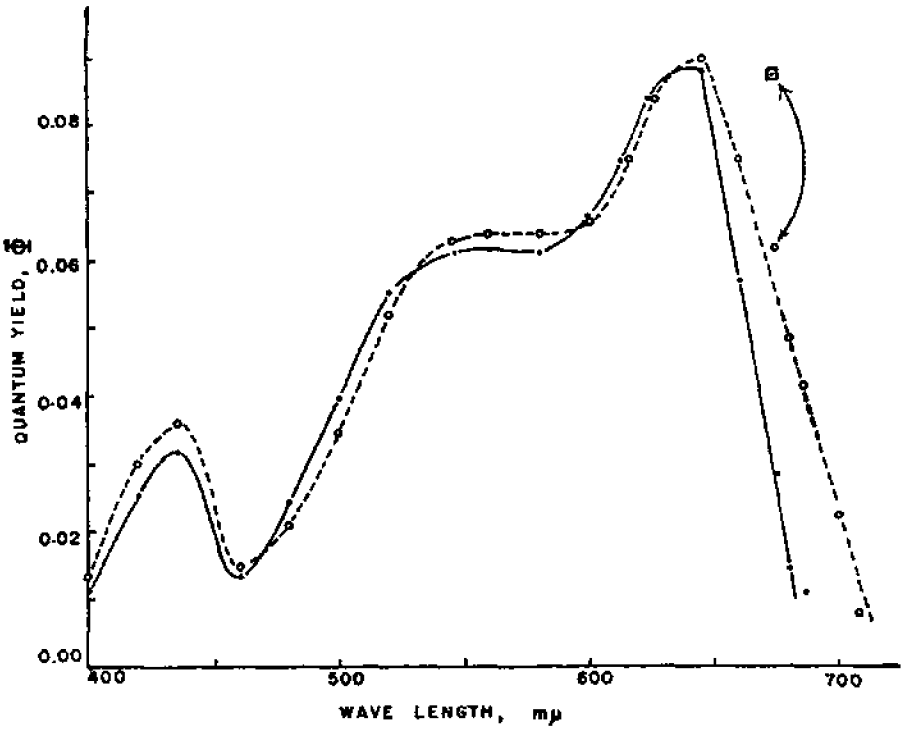

FIGURE 1. Quantum yield, Φ, as a function of wave length, for cells of *Porphyridium cruentum* cultured in white light of low intensity. The solid curve shows measurements at 20°C., the broken curve at 5°C. The point indicated by a square, and connected by an arrow to a corresponding lower point, shows the effect of supplementary light of short wave length on the yield at 675 mμ.

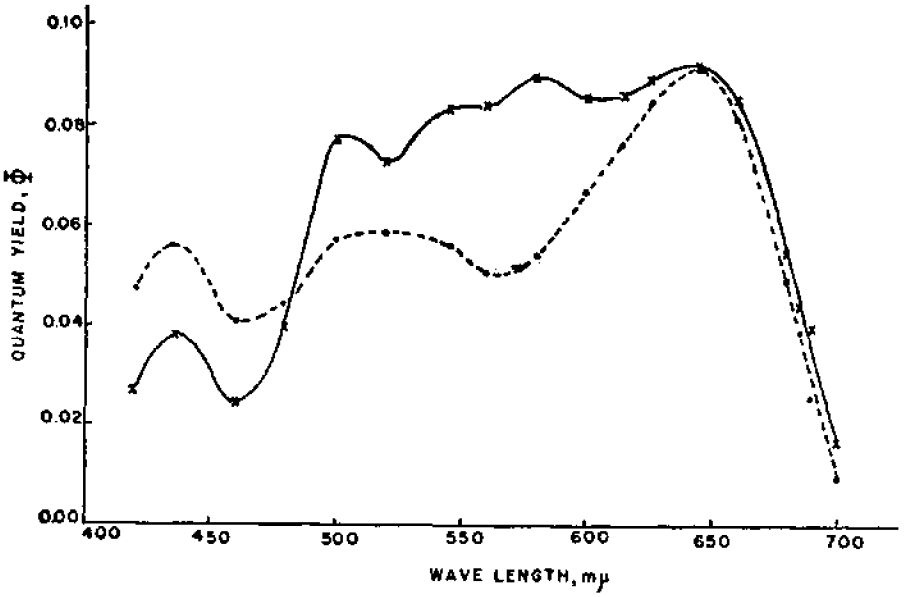

FIGURE 2. Quantum yield, Φ, as a function of wave length, for cells of *Porphyridium cruentum* grown in green light and blue light (the solid and dotted curves respectively).

in general it is lower, and often much lower. At 436 mμ, the yield is low for all types of *Porphyridium* cells which were tested. Haxo and Blinks regarded the low yields in blue as evidence of inactivity of chlorophyll, but since the fraction absorbed by carotenoids is unknown, it is difficult to evaluate the significance of the yields in blue.

The relatively high yields at 644 mμ, compared to those at 546 mμ, led us to scrutinize the curves of Haxo and Blinks, and to consider whether our evidence was in any respect contradictory to theirs. In our opinion there is no clear contradiction, since, as we explained in the introduction, 644 mμ is a region where the action spectra give uncertain evidence as to yield for light absorbed by chlorophyll. It was at longer wave lengths that Haxo and Blinks obtained their clear evidence that the yield for light absorbed by chlorophyll was low. The maxima in their action spectra of red algae near the absorption maxima of phycoerythrin do not imply that in those regions the yield was higher than at 644 mμ, but only that the absorption was stronger. The quantum yields could well have been similar to those we observed at 546 and 644 mμ.

Our subsequent experiments, in which we used light from a monochromator and tungsten lamp, and measured the quantum yield at intervals of 10 to 20 mμ through the visible spectrum, confirmed our preliminary measurements in the mercury and cadmium lines, and also confirmed the low yield at 685 mμ, which could be deduced from the work of Haxo and Blinks (and also that of Yocum and Blinks, and of Duysens). Fig. 1 shows representative curves for quantum yields as function of wave length, for cells of *Porphyridium cruentum* cultured in white light of low intensity. The solid curve shows measurements at 20°C., the broken curve at 5°C. The two are nearly identical except at longer wave lengths, where the lower temperature gives a substantial increase in yield.

Fig. 2 shows corresponding curves for cells grown in green light and blue light (the solid and dotted lines respectively). For cells grown in blue light, the yield in the green region was lower than for any other type of cells tested. On the other hand, cells grown in green light showed the highest yield encountered in this region. These cells come the nearest to matching the yield in green with the yield near 650 mμ. It may be significant that cells cultured in green light also show the highest proportion of chlorophyll to phycobilin (Brody and Emerson). Of the total light absorbed in the neighborhood of 650 mμ as much as 70 per cent may be absorbed by the chlorophyll—the highest yields for any of the various types of cells we have used for our measurements of quantum yields.

The four curves in Figs. 1 and 2 show that the region near 644 mμ, in which we made our preliminary measurements to test the activity of chlorophyll, generally gives the maximum yield obtainable with *Porphyridium*. From 650

mμ to 685 mμ the yield drops steeply, confirming the evidence from Haxo and Blinks and others that in the region of the red absorption maximum of chlorophyll the yield is very low. This steep decline in yield from 650 to 680 mμ is reminiscent of the decline reported by Emerson and Lewis (1943) for *Chlorella* photosynthesis between 685 and 700 mμ; they drew attention to the fact that the decline began at wave lengths where light absorption by chlorophyll was still strong. Probably the decline in yield in *Porphyridium* beyond 650 mμ is analogous to the decline for *Chlorella* beyond 685 mμ. The yield in the region around 680 mμ is obviously not a good criterion of the maximum efficiency of light absorbed by chlorophyll in *Porphyridium*. The yield near 650 mμ would be more representative, in spite of the uncertainty introduced by the fraction of absorption attributable to phycocyanin in this region.

We found that the extent to which the maximum yield of photosynthesis could be sustained at longer wave lengths was modifiable by temperature, and also by supplementary illumination. Both these effects have already been described (Emerson *et al.* (1956)), and are qualitatively similar for *Chlorella* and *Porphyridium*. The combination of a temperature of 5°C. and supplementary light from a mercury lamp, seems to extend maximum yield of *Porphyridium* photosynthesis about as far toward long wave lengths as the maximum yield for *Chlorella* extends. The point in Fig. 1 indicated by a square, and connected by an arrow to a corresponding lower point, shows the effect of supplementary light of short wave length on the yield at this wave length (675 mμ). The supplementary light has raised the yield for light of 675 mμ to a level equal to the maximum at 650 mμ.

Short term exposures to light of various wave lengths modify the yield of photosynthesis in specific ways, without apparent change in the concentration of pigments. In general the maximum yield at or near 650 mμ remains unchanged, and it is the yield for light absorbed by phycoerythrin which is modified by adapting light. These, and related observations on fluorescence are reported elsewhere (Brody and Brody (1959)).

DISCUSSION

In comparing our results with *Porphyridium*, with results of corresponding measurements on representatives of other algal groups, we shall regard *Porphyridium* as representative of red algae in general, just as we regard *Chlorella* as representative of green algae, *Navicula* as representative of diatoms and brown algae, and *Chroococcus* as representative of blue-greens. We may find in time that there are significant differences among individual species of each group, with respect to dependence of yield of photosynthesis upon wave length of light. We know already that different species within each group contain

different proportions of the pigments characteristic of the group, and it is to be expected that these differences will be reflected in the dependence of yield of photosynthesis upon wave length. For the moment, however, we are concerned with broad differences which may perhaps be correlated with the pigment combinations characteristic of major groups of algae. It is true that Haxo and Blinks derived their generalizations concerning the role of chlorophyll and phycoerythrin in red algae from observations on a number of species, not including *Porphyridium*, but as we mentioned earlier, the results with *Porphyridium* (both ours and those of Duysens), seem to match those of Haxo and Blinks in certain fundamental aspects. Whether *Chlorella*, *Navicula*, and *Chroococcus* are equally representative of the algal groups for which their respective pigment combinations are characteristic remains to be seen, but pending further evidence, we treat them as if they were.

Our first conclusion from the experiments with *Porphyridium* is that there is no need to suppose that the light absorbed by the chlorophyll *a* of red algae gives a lower yield of photosynthesis than light absorbed by chlorophyll *a* in other algae. The maximum yield is essentially equal for all the algal types so far investigated, regardless of the different accessory pigments which accompany the chlorophyll *a* in the different types. In every case, maximum yield extends to wave lengths where large fractions of the absorbed energy are certainly absorbed by the chlorophyll *a*, so there can be hardly any doubt that at least at these wave lengths, the yield of photosynthesis for light absorbed by chlorophyll *a* is about the same in all algae. There is, therefore, no need for a theory or hypothesis to account for a general inactivity of chlorophyll *a* in red algae.

Our results show that the yield for light absorbed by phycoerythrin varies considerably, depending upon the wave length of light used for culturing or preconditioning the cells. In general, the light absorbed by phycoerythrin is used with a quantum yield lower than the maximum observable for light absorbed by the chlorophyll *a*, but *Porphyridium* cells grown or conditioned in green light show a yield for light absorbed by phycoerythrin nearly as high as the maximum for light absorbed by chlorophyll *a*. Since it has been shown by Duysens and by French and Young (1952) and others that chlorophyll fluorescence can result from primary absorption of light by the phycoerythrin, we know that transfer of excitation energy from phycoerythrin to chlorophyll *a* is possible, and we may extend to red algae the generalization that the yield of photosynthesis from light absorbed by accessory pigments is attributable to transfer of excitation energy from accessory pigments to chlorophyll *a*. It remains possible to generalize that in all cases the chlorophyll *a* is the final receiver of the excitation energy, and the direct sensitizer of photosynthesis.

The variability of the yield of photosynthesis at wave lengths near the absorption maximum of phycoerythrin (546 mμ) may be ascribed to differences

in the efficiency of transfer of excitation energy from phycoerythrin to chlorophyll. Duysens estimated this efficiency at about 80 per cent, but our results indicate that there must be a considerable range in transfer efficiency. Correlation can be made with the proportions of phycoerythrin and chlorophyll, but this does not seem to be the only decisive factor because, as mentioned above, the yield of photosynthesis at 546 mμ seems to be easily modifiable by relatively short exposures to green or blue light, without apparent change in the ratio of the pigments.

In two cases our *Porphyridium* cells showed a yield for light absorbed by phycoerythrin practically equal to the maximum for light absorbed by their chlorophyll. In both these cases the cells had been grown in green light. However, *Porphyridium* cells grown under all other conditions of illumination always showed a lower yield for light absorbed by phycoerythrin, than for light absorbed by chlorophyll (and phycocyanin) at 644 mμ. We may compare this observation with the conclusion drawn by Emerson and Lewis (1942) that light absorbed by the phycocyanin of *Chroococcus* was used for photosynthesis at a yield practically equal to that for light absorbed by chlorophyll. (Their *Chroococcus* cells were grown in white light.) This apparent difference between the phycocyanin of *Chroococcus* and the phycoerythrin of *Porphyridium* may be attributable to the greater overlapping between the fluorescence band of phycocyanin and the absorption band of chlorophyll, and consequent greater probability of transfer of excitation energy from the phycocyanin to chlorophyll. In *Porphyridium*, comparable efficiency of transfer from phycoerythrin to chlorophyll seems to be exceptional, and to result only from cultivation of the cells in green light.

Porphyridium also resembles algae of other groups in showing a steep decline in yield of photosynthesis at longer wave lengths, but in *Porphyridium* (and certainly in the red algae studied by Blinks and coworkers as well), this decline begins at shorter wave lengths than is the case for the other three algae for which data are available (*Chlorella* (1943), and *Chroococcus* (1942), investigated by Emerson and Lewis, and *Navicula*, investigated by Tanada). The difference is so striking that it led to the suggestion (which we have seen is erroneous) that in red algae all light directly absorbed by chlorophyll *a* is utilized for photosynthesis with low quantum yield. In the red algae the steep decline from maximum yield begins at about 650 mμ, while in the other types of algae tested, maximum yield extends to 675 or 680 mμ. In all cases the decline begins within the range of light absorption by chlorophyll *a*. Even at 685 mμ, about the longest wave length at which maximum quantum yield has been reported (*Chlorella*), absorption by chlorophyll *a* is still strong. If the red absorption band of chlorophyll represents a single excited state, then absorption anywhere within the band should lead to the same excited state, and one should therefore expect the same quantum yield. Franck (1958) has suggested that two different but nearly equal electronic transitions

(a π-π and an n-π) may be included in the red absorption band. In those molecules in which the n-π is the lower one and the π-π the higher, the former transition leads to the formation of metastable triplet states, while the latter produces singlet states. Franck supposes that the maximum yield of photosynthesis requires approximately equal numbers of molecules in singlet and triplet excited states, and attributes the low yield at longer wave lengths to a deficiency of singlets. He ascribes the observed differences in extension of maximum yield toward long wave lengths in the various algal groups to differences in the water content of the layers in which the chlorophyll molecules are distributed (the water content determining the proportion of chlorophyll molecules in which the n-π state is below the π-π). It is, of course, entirely possible that there are systematic differences between red algae and algae of other types, with respect to their layers of chlorophyll molecules, and that corresponding differences in the long wave limit of maximum yield of photosynthesis in red algae and other types of algae should be ascribed to such differences. However, the fact that a steep decline from maximum yield at relatively short wave lengths seems to be characteristic of algae in which the chlorophyll *a* is accompanied by phycoerythrin, suggests an alternative possibility that the location of the decline may be connected in some way with the accessory pigment, rather than with variable properties of the chlorophyll *a* itself. Emerson *et al.* (1957) mentioned that the region of low yield at long wave lengths can generally be associated with the range of wave lengths at which light absorption can be attributed to chlorophyll *a* alone, or at which other pigments cannot be expected to contribute appreciably to absorption. Light absorption by the phycoerythrin of red algae is limited to shorter wave lengths, *i.e.* extends less toward long wave lengths, than absorption by other accessory pigments known to contribute to photosynthesis, with the possible exception of fucoxanthol. Tanada found that in the diatom, *Navicula*, maximum yield extended to about 675 mμ, much farther into the red than we could expect absorption by fucoxanthol to extend, but in *Navicula*, fucoxanthol is accompanied by chlorophyll *c*, whose absorption may well extend to about 675 mμ. Absorption by the phycoerythrin of *Porphyridium* is probably negligible at wave lengths longer than about 600 mμ, rather short of the 650 mμ region where the steep decline from maximum yield begins, but *Porphyridium* also contains phycocyanins which provide appreciable absorption probably out to 650 mμ. In *Chlorella*, absorption by chlorophyll *b* probably extends to about 680 mμ, and it is beyond this point that *Chlorella* shows its steep decline. The association of low yield with the range of wave lengths at which all or practically all light absorption is attributable to chlorophyll *a* alone, led Emerson *et al.* (1957) to suggest that the accessory pigments may make some contribution to photosynthesis more specific than the mere transfer of excitation energy to the chlorophyll *a*. The obvious weakness of this suggestion is that it fails to account for a long

wave decline in the yield of chlorophyll fluorescence, reported by Duysens to correspond approximately with the decline in yield of photosynthesis. It is not evident how lack of excitation of an accessory pigment could have an adverse effect on yield of fluorescence from light absorbed by chlorophyll *a*.

Lavorel (1957) suggested that there may be an absorption band for chlorophyll *a* on the long wave side of 685 mμ, which he attributed to a dimer, since evidence for it appeared only in very concentrated solutions. He suggested that absorption by the dimer might account for the diminished yield at long wave lengths. S. Brody (1958) has reported a new excited state of chlorophyll, just below the singlet state associated with the main red absorption maximum. He attributes this lower excited state to an aggregated form of chlorophyll (perhaps Lavorel's dimer), and suggests that it may account for the low yield of photosynthesis at long wave lengths. It remains to be seen whether differences in proportions of aggregated and unaggregated chlorophyll can account for the difference between *Chlorella* and *Porphyridium* with respect to extension of maximum yield toward long wave lengths.

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